Expression of EMMPRIN/CD147 and Ki-67 in oral squamous cell carcinoma:An immunohistochemical study

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Abstract: Background Oral squamous cell carcinoma (OSCC) is the most frequent malignancy of the oral cavity, highly invasive with unfavorable prognosis and unimproved 5-year survival rate for at least two decades. An elevated level of EMMPRIN in cancer tissues have been correlated with tumor invasion in numerous cancers including oral cavity and larynx. Ki-67is a specific marker of proliferation and the expression of which is strictly associated with cell proliferation and used to measure the growth fraction of cells in human tumors. **Objectives:** The aim of this study was to evaluate the immunohistochemical expression of EMMPRIN/CD147 and Ki67 markers in OSCC and to correlate the expression of either marker with each other and with the clinico-pathological parameters of OSCC. **Methods:** Thirty five formalin-fixed, paraffin- embedded tissue blocks of OSCC were included in this study. H&E stain was done for each block for reassessment of histological examination. The expressions of EMMPRIN and Ki67 were detected by immunohistochemical method. **Results:** The expression of EMMPRIN and Ki-67 were positive in all OSCC cases. EMMPRIN high expression score was observed in 26 cases (74.3%). No significant relationships were found between clinicopathologic factors and this protein except for clinical stage, and histological grade (P=0.047 and 0.005 respectively). The highexpression of EMMPRIN was associated with higher grade and advanced stage of OSCC. On the other hand, Ki67 with high proliferation score was observed in 17 cases (48.5%) of 35 OSCC. Significant relationship was found between Ki67 and histologic grade (P=0.003).Also a statistically significant correlation was found between EMMPRIN high expression and Ki67 high proliferation scores (P=0.001). **Conclusion:** Increased expression of EMMPRIN in more than two third of cases with statistical significant association with proliferative marker highlight the importance of EMMPRINin cancer progression, indicating that EMMPRIN couldbe an attractive target for immunotherapeutic approaches in a group of patients with OSCC.

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Key words: EMMPRIN/CD147; Ki67; Oral squamous cell carcinoma; immunohistochemistry

1. Introduction

Head and neck cancer is the sixth most common cancer, representing 3% of all localizations. In 48% of these cases, the tumors were located in the oral cavity and 90% are squamous cell carcinomas **(Jemal *et al.*, 2009 & Olimid *et al.*,2012).**

Oral squamous cancer cell carcinoma (OSCC) ranks among the top ten most frequently cancers, and is highly invasive with bad prognosis; despite the recent advances in cancer therapy, the 5-year survival rate of patients has remained at < 50% **(Specenier and Vermorken, 2010)**. Little is known about of the molecular events that govern OSCC initiation, progression and metastasis. Development of OSCC is a complex and multistep process, with transformation from oral premalignant dysplastic lesion to OSCC. Progression is generally known to involve the intervention of proteinases **(Siqueira *et al.*, 2010).**

During SCC progression, subsets of SCC cells undergo an epithelial-to-mesenchymal transition (EMT) to become highly invasive. The extracellular matrix metalloproteinase inducer (EMMPRIN) contributes to EMT by activating local matrix metalloproteinase (MMPs) **(Siu *et al.*, 2013)** and through transforming fibroblasts to cancer associated fibroblasts (CAFs) **(Xu *et al.*,3013).**

Extracellular matrix metalloproteinase inducer (EMMPRIN), is a transmembrane glycoprotein of the immunoglobulin superfamily, also known as CD147, has been identified as a tumor-cell membrane protein that stimulates (MMP) production in stromal fibroblasts. EMMPRIN is overexpressed in various tumor cells including those in head and neck carcinoma, and is also known to promote tumor invasion and lymph node metastasis **(Yang *et al.*, 2013).**

It is well understood that transition of the normal oral epithelium to dysplasia to malignancy is featured by increased cell proliferation. Discovery of various proliferation markers has enabled the detection of the hyperactive state of the epithelium, the basal layer is the only proliferative compartment for normal oral epithelium, and hence, any sign of proliferative cellular activity beyond the basal layer

should be considered as a warning sign **(Dwivedi *et al.*, 2013)**.

Ki-67 is a nuclear protein expressed in the G2- and M-phases of actively dividing cells. This antigen is a proliferation marker that correlates with the presence and severity of epithelial dysplasia. It provides significant information about the degree of aggressiveness and prognosis of (OSCC) (**Patel *et al.*, 2014).**

The purpose of this study was to evaluate the immunohistochemical expression of EMMPRIN/CD147 and Ki67 markers in oral squamous cell carcinoma and to correlate the expression of either marker with each other and with the clinico-pathological parameters of OSCC.

2. Patients and Methods

This retrospective study included 35 biopsies of OSCC were retrieved from archives of Histopathology laboratory at AL-Zahraa hospital, Al-Azharuniversity, Cairo, Egypt andprivate laboratory, duringthe period from 2008 -2011. All cases were selected on the basis of availability of paraffin blocks with sufficient amount of tissue for re-cut, histopathological re-examination and immunohistochemical staining. As the material of this study was archival paraffin blocks with no direct contact to the patient, who were unknown to us, there is no need for patient approval or consent. The clinical data related to the selected cases were prospectively collected in a computerized database.

Histopathologicalexamination:

All specimens were formalin- fixed, routinely processed, and embedded in paraffin. Three 5-um thick sections were prepared from each tissue block, one of them stained with hematoxylin and eosin forhistopathological examination to confirm the diagnosis of cases. Tumor stage was classified according to the 7th edition of the classification of malignant tumors of American Joint Committee on Cancer **(Brandwein-Gensler & Smith, 2010)** and graded according to the **WHO** classification **(2005)** into well-differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3) OSCC **(Barnes *et al.*,2005).** In addition, evaluation of lymph node metastasis (negative or positive), surgical margin, perineural and lymphatic invasion were carried out.

Immunohistochemical study

a)Immunohistochemical staining

Two sections were prepared from each case on positively charged slides and subjected to immunohistochemical staining using the streptavidin – biotin alkaline phosphate methods for expression of monoclonal antibodies for EMMPRIN (mouse monoclonal sc-21746 antibody; dilution 1:100; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and Ki67 (mouse monoclonal sc-21743 antibody; dilution 1:50; Dako-cytomation). The sections were placed in an oven at 50 ◦C for 30 min and were deparaffinizedinxylene, rehydrated in gradedalcohol dilution, washed in PBS,incubatedwith 0.3% hydrogen peroxide to block endogenous peroxidase activity, washed in PBS again and boiled in citrate buffer solution (pH 6.0) using a microwave for 10 min at 60◦C for antigen retrieval. After cooling at room temperature, the sections were incubated with primary antibody overnight in a humidified chamber.Rinsed with PBS, the sections were incubated for 30 min at 37 with biotinylated secondary antibody and streptavidin conjugated to horseradish peroxidase, respectively. After three rinses with PBS, the sections were incubated with diaminobenzidine substrate, then rinsed with distilled water and counterstained with hematoxylin.

Positive control for two antibodies was normal buccal mucosa.Negative controls were prepared by omitting the primary antibody under identical test condition.

b) Interpretation of immunohistochemical staining

EMMPRIN (CD147) scoring

Cytoplasmic and membranous staining for EMMPRIN was accepted as positive. Each slide was evaluated according to staining extent and intensity. The extent of staining was calculated as the percentage of stained cells and was scored semiquantitatively, using a 0 to 4 scale for expression, where 0 = no expression, 1 = 1-25%, 2 = 26-50%, 3 = 51-75% and 4 = 76-100%, staining intensity was also categorized into three groups, where 1 = weak, 2 = moderate and 3 = strong. Staining extent and intensity scores were added to give combined scores that were then allocated to 4 groups in which the categories were: 0-1, negative staining; 2-3, weak staining; 4-5, moderate staining; and 6-7, strong staining **(Kefeli *et al.*, 2010).**

For statistical purposes, staining in cancer cells was further divided into two groups; low expressionandhigh expression scoresaccording to (score 2-3 and 4-5) and (score 6-7) respectively.

Ki-67 scoring

Semiquantitative evaluation marking was performed in accordance with (**Maheshwari *et al.*, 2013)** in this respect, presence of brown precipitate at the site of target antigen (nucleus) was indicative of positive immunoreactivity. Those histological section with uniform and good intensity staining were assessed for scoring from 1-3.

1- +++ - High proliferation- >50% positive cells.
2-++- Moderate proliferation- 30-50% positive cells.
3- +- Low proliferation -10-30% positive cells.

Statistical analysis:

Data were collected, revised, coded and entered to the statistical package for social science (SPSS) version 17. The qualitative data were presented as number and percentages and compared together using Chi-square test while the quantitative data were presented as mean, standard deviations and ranges and the comparison between more than two groups were done by using One Way Analysis of Variance (ANOVA) test. The confidence interval was set to 95 % and the margin of error was set to 5%. Differences were considered statistically significant when *P* is 0.05 or less, highly significant when *P* is less than 0.01 and not significant when *P* is more than 0.05.

3. Results

Clinicopathological results

This study was carried out on 35 OSCC cases. The age of patients range from 22 to 75, the most of the cases 24 (68.6%) aged were above 60 years with males predominance 25/35 (71.4%). The most common site was the tongue 14/35 cases (40%). Clinicopathological data of the studied cases are displayed in **Table(1).**

Table 1 – Clinicopathological parameters of 35 OSCC

Clinicopathologic parameters		Frequency	Percent%
Age	Age > 60 years	24	68.6%
	Age < 60 years	11	31.4%
Gender	Female	10	28.6%
	Male	25	71.4%
Site	Check	7	20%
	Floor	6	17.1%
	Hard palate	3	8.6%
	Lip	5	14.3 %
	Tongue	14	40 %
Stage	Stage I	5	14.3%
	Stage II	13	37.1%
	Stage III	5	14.3%
	Stage IV	12	34.3%
Histologic grade	Grade I	15	42.8%
	Grade II	10	28.6%
	Grade III	10	28.6%
LNmetastasis	Negative	20	57.2%
	Positive	15	42.8%
Surgical margin	Negative	20	57.2%
	Positive	15	42.8%
Perineural invasion	Negative	25	71.4%
	Positive	10	28.6%
Lymphatic invasion	Negative	27	77.1%
	Positive	8	22.9%

Immunohistochemical results

EMMPRIN expression and its correlation with clincopathologic factors

All studied cases of OSCC (100%) showed positive EMMPRIN immunoreactivity with variable amounts ranging from low to high expression.In normal oral mucosa, staining was localized to the keratinocyte cell membrane with a slightly enhanced reactivity in the basal cell layer. In tissue sections of OSCC, antibodies to EMMPRIN reacted with the cell membrane throughout the entire specimen. Cytoplasmic and membranous staining pattern were acquired by tumor cells,also expressed in the adjacent connective tissue cells mainly fibroblasts and endothelial cells. Twenty six (74.3%) cases were with high expression and 9 (25.7%) caseswere with low expression scores. Correlation of EMMPRIN immunohistochemical expression with different clinicopathological variables in the studied cases showed a significant positive associations between EMMPRIN high expression with pathological stage and tumor grade (*P*=0.047 and 0.005 respectively). EMMPRIN expression was found to be significantly associated with an increasing invasiveness of the tumor. Similarly,low-grade tumors (G1) were associated with low EMMPRIN expression scores, whereas high-grade tumors (G2 and G3) showed higher expression scores. However no significant correlation with other clinicopathologic parameters ***(Table 2) (Fig 1, A- D).***

Table 2-Correlation between EMMPRIN/CD147 expression, andclinicopathological characteristics in OSCC cases

		EMMPRIN (CD147)				Chi-square test	
		Low Expression score		High expression score		X^2	*P*-value
		No.	%	No.	%		
Age	Age>60 years	7	77.8%	17	65.4%	0.476	0.490
	Age< 60 year	2	22.2%	9	34.6%		
Gender	Female	3	33.3%	7	26.9%	0.0037	0.951
	Male	6	66.7%	19	73.1%		
Site	Check	3	33.3%	4	15.4%	4.088	0.394
	Floor	2	22.2%	4	15.4%		
	Hard palate	0	0.0%	3	11.5%		
	Lip	0	0.0%	5	19.2%		
	Tongue	4	44.4%	10	38.5%		
LN	Negative	5	55.6%	15	57.7%	0.012	0.911
	Positive	4	44.4%	11	42.3%		
Surgical margin	Negative	7	77.8%	13	50.0%	2.106	0.147
	Positive	2	22.2%	13	50.0%		
Perineural invasion	Negative	7	77.8%	18	69.2%	0.239	0.625
	Positive	2	22.2%	8	30.8%		
Lymphatic invasion	Negative	8	88.9%	19	73.1%	0.948	0.330
	Positive	1	11.1%	7	26.9%		
Stage	Stage I	3	33.3%	2	7.7%	7.939	**0.047**
	Stage II	4	44.4%	9	34.6%		
	Stage III	2	22.2%	3	11.5%		
	Stage IV	0	0.0%	12	46.2%		
Grade	Grade I	8	88.89%	7	26.9%	10.744	**0.005**
	Grade II	1	11.11%	9	34.6%		
	Grade III	0	0.00%	10	38.5%		

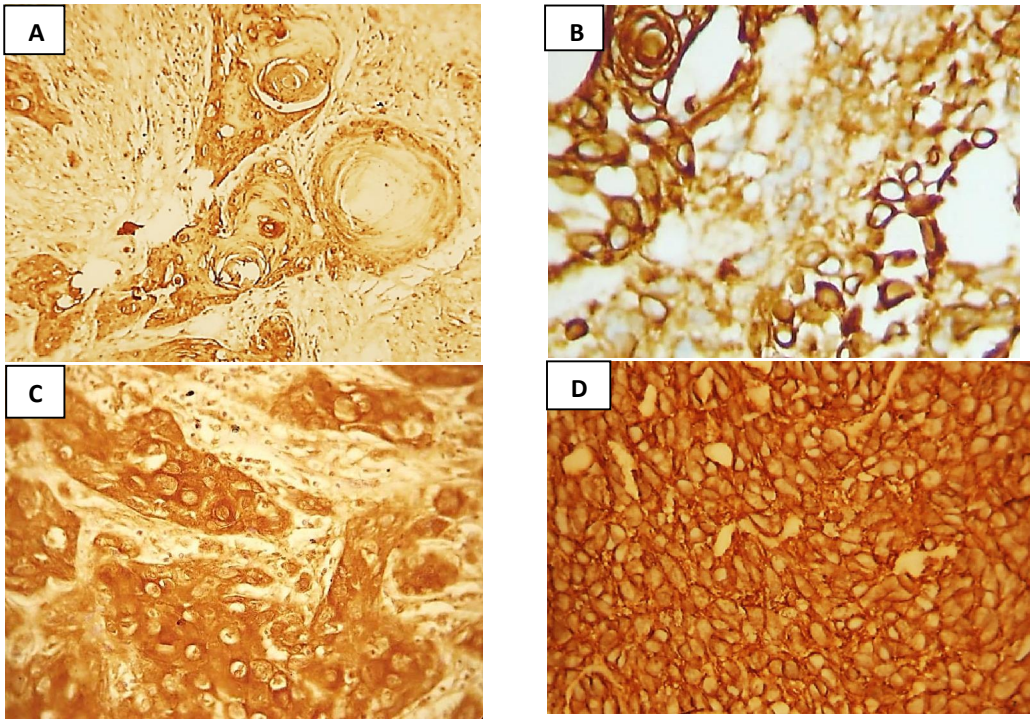


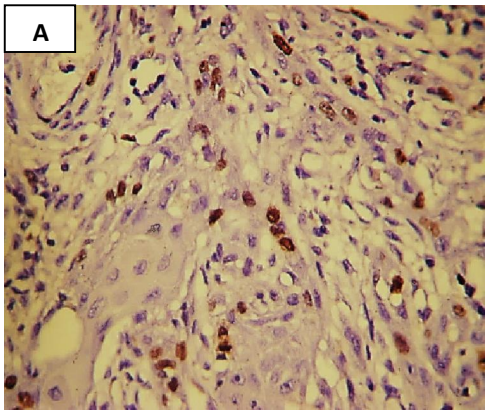
Fig.(1): **Immunohistochemical staining of EMMPRIN/CD147 expression in OSCC tissues. (A, B)Low focal expression EMMPRIN score in grade I OSCC (X200, 400). (C)High diffuse EMMPRIN expression score in grade II OSCC with peritumoral fibroblasts staining(X200). (D) High membranous EMMPRIN expression score in grade III OSCC (X400).**

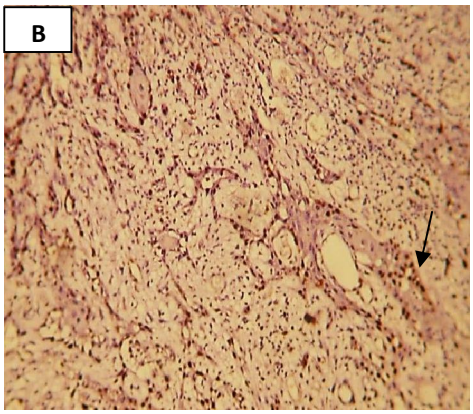
Ki-67 expressionand its correlation with clincopathologic factors

Ki-67 expression was identified at nuclear level in all analyzed cases. Eighteen cases (51.4%) were with low and moderate proliferation scores and 17 cases (48.6%) were with high proliferation score presenting high intensity at level of invasive front. Statistical analysis demonstrated a significant positive association between high Ki-67 immunostaining scores and tumor grade(P = 0.003) i.e. the more the increase of the tumor grade, the higher the Ki-67 expression score. The Ki-67 staining pattern in these poorly differentiated tumors showed a more uniformdistribution, as compared with the stratification revealed by the Ki-67 pattern in the well-differentiatedtumors. No significant associations were detected between Ki-67 expression and other clinicopathological characteristics. (***Table 3)*** **(Figs 2, A-D)**.

Table 3- Correlation between Ki-67 expression andClinicopathological characteristics in OSCC cases

		Ki67				Chi-square test	
		Low+Moderate proliferation score		High proliferation score			
		No.	%	No.	%	X^2	*P*-value
Age	Age > 60 years	11	61.1%	13	76.5%	0.957	0.328
	Age< 60 years	7	38.9%	4	23.5%		
Gender	Female	5	27.78%	5	29.4%	0.072	0.789
	Male	13	72.22%	12	70.6%		
Site	Check	3	16.7%	4	23.5%	1.792	0.774
	Floor	3	16.7%	3	17.6%		
	Hard palate	1	5.6%	2	11.8%		
	Lip	2	11.1%	3	17.6%		
	Tongue	9	50.0%	5	29.4%		
LN	Negative	10	55.6%	10	58.8%	0.038	0.845
	Positive	8	44.4%	7	41.2%		
Surgical margin	Negative	10	55.6%	10	58.8%	0.038	0.845
	Positive	8	44.4%	7	41.2%		
Perineural invasion	Negative	11	61.1%	14	82.4%	1.933	0.164
	Positive	7	38.9%	3	17.6%		
Lymphatic invasion	Negative	13	72.2%	14	82.4%	0.509	0.476
	Positive	5	27.8%	3	17.6%		
Stage	Stage I	4	22.2%	1	5.9%	4.986	0.173
	Stage II	6	33.3%	7	41.2%		
	Stage III	4	22.2%	1	5.9%		
	Stage IV	4	22.2%	8	47.1%		
Grade	Grade I	12	66.67%	3	17.65%	11.781	0.003
	Grade II	5	27.78%	5	29.41%		
	Grade III	1	5.56%	9	52.94%		





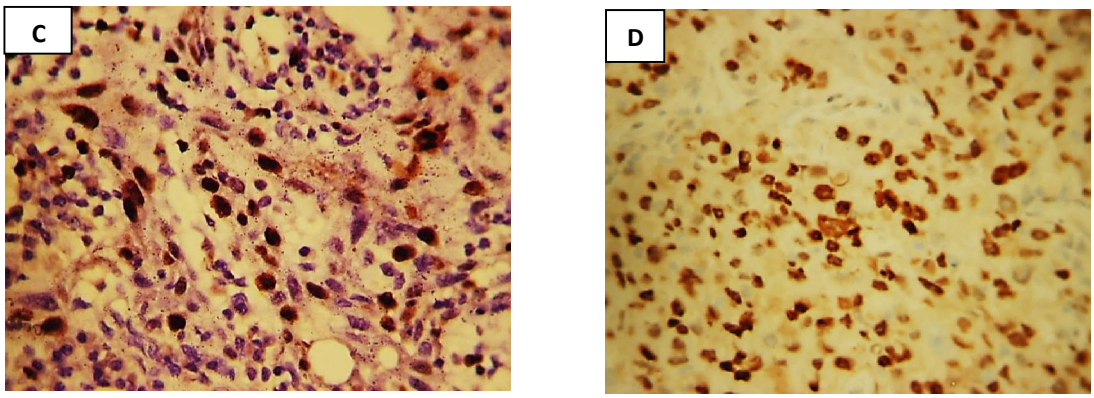


Fig.(2): Immunohistochemicalstaining of Ki67/MIB1 in OSCC tissue.(A) Ki -67moderateproliferation score in grade I OSCC. The carcinoma cells in the periphery of the nests stain strongly for Ki-67 more than in the center of the nests. (X 200). (B)Ki-67 expression at level of invasive front (arrow)(X100).(C),(D) Ki-67 high proliferation score in grade III OSCC. Carcinoma cells form small clusters or nests without differentiation or keratin pearls. The carcinoma cells in the nests are diffusely stained strongly for Ki-67. The tumor stroma is negative for Ki-67 staining(x400).

Correlation between EMMPRIN and Ki 67 expression in OSCC

The correlation between EMMPRIN and Ki67 immunostaining scores is shown in **Fig. 3**. Overall, a significant positive correlation was observed between Ki67 and EMMPRIN expression in OSCC(*P*=0.001). Most of cases with EMMPRIN high expression scores presented with high Ki67 proliferation scores ***(Table 4).***

4. Discussion

Oral squamous cell carcinoma (OSCC) is the most frequent type of cancer of the head and neck area. Better understanding of the molecular mechanisms regulating tumor invasion and metastasis for OSCC may lead to more effective treatment options ***(Huang et al., 2014)***. In the current work, it has been chosen as a subject of study.

Table. 4-Correlation between EMMPRIN and Ki 67 expression in OSCC

EMMPRIN (CD147) Scoring	Ki67 scoring				Chi-square test	
	Low& Moderate proliferation		High proliferation			
	No.	%	No.	%	X^2	*P*-value
Low expression	9	50.0%	0	0.0%	11.442	0.001
High expression	9	50.0%	17	100.0%		
Total	18	100.0%	17	100.0%		

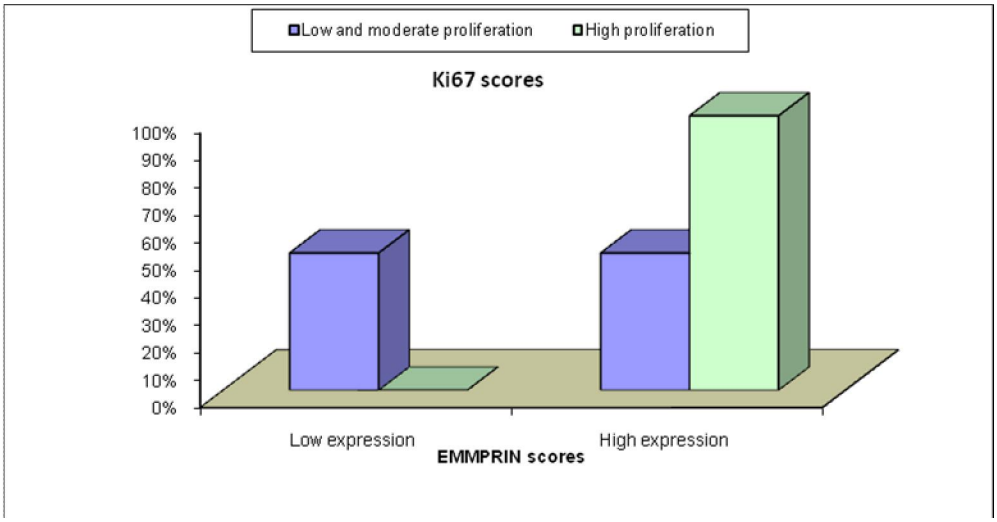


Fig. 3: Significant positive correlation between EMMPRIN and Ki-67expression scores in OSCC.

Oral carcinogenesis is a multistep phenomenon whose progression is segregated into the early and late stages. Earliest morphological changes are potentially malignant oral lesions of leukoplakia and erythroplakia with dysplasia. A series of molecular events bring about this progression from normal to dysplasia to carcinoma in situ to invasion and eventual metastasis, that is associated with controlled proteolysis, and involves interactions between the tumor cells and the extracellular matrix (ECM) mediated by various soluble and cell surface molecules including EMMPRIN **(Shrestha *et al.*, 2014).** The role of EMMPRIN in tumor growth and invasion was illustrated by the accelerated growth and increased invasiveness of EMMPRIN-overexpressing human breast cancer cells **(Lescaille*et al.*,2012).**

Being an important factor in epithelial-connective tissue interactions, EMMPRIN might be involved in OSCC progression and metastasis, so this study has been chosen to evaluate expression of EMMPRIN in OSCC and correlate with cellular proliferation and clinicopathologic parameters.

In the current work, all the examined cases of OSCC showed positive EMMPRIN immunoreactivity with high expression in more than two-thirds of the cases (74.3%).This suggests that EMMPRIN play an important role in oral tumor progression. Similarly, the high EMMPRIN positivity has been reported in OSCC by **(Monteiro *et al.*,2014)**, andmany cancers including; hepatocellular carcinoma **(Zhang *et al.*, 2007)**, cervical cancer **(Ju*et al.*,2008)** and lung adenocarcinoma **(Sienel *et al.*,2008).** Lower expression rate has been reportedby **Huang *et al.* (2009)** in tongue squamous cell carcinomas (67%), **Zhang *et al.*(2012)** in OSCC(65.71%), also reported by **Gou *et al.*(2014**) in laryngeal carcinoma (87.5%).

In the present study, EMMPRIN showed cytoplasmic and membranous staining patterns for the positive cells. This result is in accordance with studies done by **Piao *et al.*(2012) andYang *et al.*(2013)** who examined its immunohistochemistry in hypopharyngeal carcinoma and salivary duct carcinoma respectively. This expression pattern can be attributed to EMMPRIN'S structure, as it has a cytoplasmic and membranous domain which was previously described by **Iacono *et al.*(2007).**

Our results showed EMMPRIN expression was significantlyassociated with histological grade. Moderate and poorly differentiated tumors showed EMMPRIN high expression than well differentiated tumors. This result is supported bystudy done by **Monteiro *et al.* (2014)** whoobserved that the expression of EMMPRIN protein in well-differentiated tumors was lower than that in moderately and poorly differentiated tumors in OSCC. Also in accordance with **Feng *et al.*, 2013** who foundthe same association in esophageal cancer, colon cancer, cervical cancer, lung cancer, ovarian cancer, and hepatocellular carcinoma. These observationsstrongly suggest that the EMMPRIN might be actively involved in the growth, invasion and metastasis of OSCC. Furthermore, the measurement of EMMPRIN levels may assist in predicting patients' prognosis. Conflicting result done by **Huang *et al.* (2009)** in tongue SCC.

In the current study we observed that EMMPRIN high expression was noted with advanced clinical stage. This result is in line with **Huang *et al.* (2009),** emphasizingthat EMMPRIN, plays a crucial role in tumor progression, invasion and metastasis in head and neck squamous cell carcinoma soit may represent a useful biomarker for prognostic evaluation.Uncontrolled proliferation of cells is one of the most important biological mechanisms associated with oncogenesis, a number of studies indicating that proliferation has prognostic value in a variety of tumors.Ki67 is one of the mitotic indicators in proliferative activity of tumors. Expression of Ki-67 in mean of proliferative activity of tumor cells is one of the indicators for tumor invasion potential and invasive activity of cancers related to degree of malignant neoplastic cells **(Motta *et al.*,2009)**.In our study, all the cases showed Ki-67 expression. A similar observation was reported by **Raju*et al.* (2005)**; **Dragomir *et al.*(2012).** The present work showed a statistically significant relationship between Ki-67 and tumor grading. The highest Ki-67 expression was found in poorly differentiated squamous cell carcinoma and presenting high intensity at the tumoral invasion front which is the most important area for prognostic determination of oral cancer. It consists of many molecular and morphological characteristics that reflect tumor progression better than other parts of the tumor. Several molecular events of importance for tumor spread such as gains and losses of adhesion molecules, secretion of proteolytic enzymes, increased cell proliferation and initiation of angiogenesis occur at the invasive front. Results similar to those found in this study was reported in previous studies in OSCC **(ARUL *et al.*, 2011; Humayun** and **Prasad, 2011; Maheshwari *et al.*, 2013; Dwivedi *et al.*, 2013)**. The expression of Ki-67 is correlated with the grading of OSCC as it depicts the growth fraction of the tumor and its proliferative status increased according to the aggressiveness of the tumor. Conflicting results on the relationship of Ki67 with OSCC grade observed by studies done by **Roland *et al.* (1994)**; **Bettendorf & Herrmann (2002).**

Hence, the present study suggests that the Ki-67 expression in OSCC is significant and useful in predicting histologic grade of differentiation,prognosis of the lesion and determining survival rates. To

explore the mechanism about the regulatory effect of EMMPIN on tumor growth, we examined expression of Ki-67, a good proliferation marker and consequently found a significant positive correlation between the two parameters, high EMMPRIN expression has been related to high Ki-67 scoring (p= 0.001) suggesting that increased EMMPRIN expression in cancer cells might be associated with adverse disease outcome. Similar results shown by previous studies (**Yang *et al.*,2010; Zhao *et al.*, 2013; Monteiro *et al.*,2014). Yang *et al.* (2006)** reporteda mechanism by which EMMPRIN provides resistance of cancer cells to anoikis, a form of apoptosis induced by loss of matrix or cellular attachment and often defective in tumor cells that are of epithelial origin and show that the effect of EMMPRIN appears to result from MAP kinase-mediated down-regulation of a BH3-only proapoptotic protein, Bim. **Marieb *et al.* (2004)** documented that upregulated EMMPRIN expression stimulates hyaluronan production by elevating hyoluronan synthases, which is closely related to the anchorage-independent growth of cancer cells**.** These results supported the opinion that EMMPRIN might enhance tumor growth of carcinoma by disrupting the balance between apoptosis and proliferation.Silencing EMMPRIN in head and neck squamous carcinoma (HNSCC) cells was shown to result in significant suppression of tumor growth **(Lescaille *et al.*,2012).**

In conclusion, this study showed increased expression of EMMPRIN in more than two third of caseswith statistical significant association with proliferative marker. This finding highlight the importance of EMMPRIN in cancer progression, indicating that EMMPRIN couldbe an attractive target for immunotherapeutic approaches in a group of patients with OSCC and could thus be considered as an objective and effective marker to predict the invasion and prognosis of OSCC.Further functional studies are needed to clarify the efficacy of anticancer therapy targeting these signal factors.

References:

1. ArulJK, Solomon J, Santhi SV. Immunohistochemical evaluation of Bcl-2 and Ki-67 in varying grades of oral squamous cell carcinoma. Journal of Scientific and Industerial Research. (70): 923-928, 2011.
2. Barnes L, Eveson JW, Reichart P, Sidransky D. World Health Organization classification of tumours: Pathology and genetics of head and neck tumours. Lyon: IARC. Press.177-80,2005.
3. Bettendorf O and Herrmann G: Prognosticrelevance of Ki-67 antigen expression in 329 cases of oral squamous cellcarcinoma. ORL J Otorhinolaryngol Relat Spec. 64(3): 200-205, 2002.
4. Brandwein-Gensler M and Smith RV.Prognostic indicators in head and neck oncology including the new 7th edition of the AJCC staging system.Head and Neck Pathology.4(1) 53–61, 2010.
5. Dragomir LP, Simionescu C, Mărgăritescu C, StepanA, Dragomir IM, Popescu MR. P53, p16 and Ki67 immunoexpression inoralsquamouscarcinomas. Rom J Morphol Embryol; 53(1):89-93. 2012.
6. Dwivedi N, Chandra S, Kashyap B, Raj V, and Agarwal A. Suprabasal expression of Ki-67 as a marker for the severity of oral epithelial dysplasia and oral squamous cell carcinoma. ContempClin Dent. 4(1): 7–12, 2013.
7. Feng L, Zhu S, Zhang Y, Li Y, Gong L, Lan M,Han X, Yao L, Zhang W. Xi Bao Yu Fen ZiMian Yi Xue Za Zhi. Expression and clinical significance of HAb18G/CD147 in malignant tumors. Xi Bao Yu Fen ZiMian Yi XueZaZhi 29(9):958-61. 2013.
8. Gou X, Chen H, Jin F, *et al.* Expressions of CD147, MMP-2 and MMP-9 in laryngeal carcinoma and its correlation with poor prognosis.Pathology & Oncology Research. 20(2):475–481,2014.
9. Huang Z, Huang H, Li H, Chen W, Pan C. EMMPRIN expression in tongue squamous cell carcinoma.Journal of Oral Pathology and Medicine.38(6):518–523, 2009.
10. Huang Z, Tan N,Guo W, Wang L, Li H, Zhang T, Liu X, Xu Q, Li J, Guo Z. Overexpression of EMMPRIN Isoform 2 Is Associated with Head and Neck Cancer Metastasis.9 (4):1-10, 2014.
11. Humayun S and Prasad VR. Expression of p53 protein and ki67 antigen in oral premalignant lesions and oral squamous cell carcinomas: An immunohistochemical study. Natl J Maxillofac Surg.2:38-46. 2011.
12. Iacono K, Brown A, Greene M, and Saouaf S. CD147 immunoglobulin superfamily receptor function and role in pathology. ExpMolPathol, 83(3): 283-295, 2007.
13. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ.Cancer Statistics. CA Cancer J Clin, 59(4):225–249, 2009.
14. Ju XZ, Yang JM, and Zhou XY. EMMPRIN expression as a prognostic factor in radiotherapy of cervical cancer. Clinical Cancer Res, 14(2): 494-501, 2008.
15. Kefeli M, Sengul AT, Yilidizl L, Baris S, Basoglu A, Kandemir B. EMMPRIN andFascin expression in non small cell lung carcinoma. Cent.Eur. J. Med. 5(6):659-665, 2010.
16. Lescaille G, Menash S, Balloy B, Khayati F, Quemener C, Podgorniak M, *et al.* EMMPRIN/CD147 up-regulates urokinase-type plasminogen activator: implications in oral tumor progression. BMC Cancer, 12:115, 2012.
17. Maheshwari V, Sharma SC, Narula V, Verma S, Jain A, Alam k. Prognostic and predictive impact of Ki67 in premalignant and malignant squamous cell

lesion of oral cavity. Int J. of Head and Neck Surgery.(4) 2:61-65. 2013.
18. Marieb EA,Zoltan-Jones A, Li R, Misra S,Ghatak S, Cao J. EMMPRIN promotes anchorage independent growth in human mammary carcinoma cells by stimulatinghyaluronan production. Cancer Res; 64:1229 – 32. 2004.
19. Monteiro LS, Delgado ML, Ricardo S, Garcez F, Amaral BD, Pacheco JJ, Lopes C,andBousbaa H.EMMPRIN Expression in Oral Squamous Cell Carcinomas: Correlation with Tumor Proliferation and Patient Survival. Biomed Res Int. 2014 (2014):905680, 9 pages. 2014.
20. Motta RD, Zettler CG, CambruzziE,Jotz GP, Berni RB. Ki-67 and p53 correlation prognostic value in squamous cell carcinomas of the oral cavity and tongue. Braz. J. Otorhinolaryngol. 75(4), 2009.
21. Olimid DA, Simionescu CE, Margaritescu CL, Florescu A. Immunoexpression of Ki67 and cyclin D1in oral squamous carcinomas. J Morphol Embryol, 53(3):795–798, 2012.
22. Patel SM, Patel KA, Patel PR,GamitB,Hathila RN and GuptaS. Expression of p53 and Ki-67 in oral dysplasia and Squamous cell carcinoma: An immunohistochemical study. International Journal of Medical Science and Public Health. 3 (10), 1201-1204. (2014).
23. Piao S, Zhao Z, Guo F, Xue J, Yao G, Wei Z, Huang Q, Sun S, Zhang B. Increased expression of CD147 and MMP-9 is correlated with poor prognosis of salivary duct carcinoma.Cancer Res. Clin Oncol. 138 (4):627-635, 2012.
24. Raju B, Mehrotra R, Oijordsbakken G, Al-Sharabi AK, Vasstrand EN, Ibrahim SO. Expression of p53, Cyclin D1and Ki-67 in Pre-malignant and Malignant Oral Lesions: Association with Clinicopathological Parameters. Anticancer Res. 25:4699-706, 2005.
25. Roland NJ, Caslin AW, Bowie GL, Jones AS. Has the cellular proliferation marker Ki-67 any clinical relevance in squamous cell carcinoma of the head and neck. ClinOtolaryngol Allied Sci; 19: 13-8. 1994.
26. ShresthaB,Subedi S, Bajracharya D, Radhakrisnan. Immunohistochemical expression of MMP-2in an experimental progression model of oral cancer. Journal of Chitwan Medical College. 4(8): 3-8, 2014.
27. Sienel W, Polzer B and Elshaki K. Cellular localization of EMMPRIN predicts prognosis of patients with operable lung adenocarcinoma independent from MMP-2and MMP-9. Mod Pathol, 21(9):1130-1138, 2008.
28. Siqueira AS, Gama-de-Souza LN, Arnaud MV, Pinheiro JJ, Jaeger RG. Laminin-derived peptide AG73 regulates migration, invasion, and protease activity of human oral squamous cell carcinoma cells through syndecan-1 and beta1 integrin. Tumor Biol, 31(1):46-58, 2010.
29. Siu A, Chang J, Lee C, Lee S, Lee C, Ramos DM. Expression of EMMPRIN modulates mediators of tumor invasion in oral squamous cell carcinoma. J Calif Dent Assoc. 41(11):831-8, 2013.
30. Specenier P, Vermorken JB. Advances in the systemic treatment of head and neck cancers. CurrOpinoncol. 22(3):200-205, 2010.
31. Xu, J, Lu Y,QiuS, Chen Z and Fan Z. A novel role of EMMPRIN/CD147 in transformation of quiescent fibroblasts to cancer associated fibroblasts by breast cancer cells. Cancer Letters.335:380-386, 2013.
32. Yang JM, Neill PO, Jin W, Foty R, Medina DJ, Xu Z, Greg Arndt M., Tang Yi, Nakada M, Yan L, and Hait WN. Extracellular matrix metalloproteinase inducer (CD147) confers resistance to breast cancer Cells to Anoikis through Inhibition of Bim. J Biol Chem. 7;281(14):9719-27 2006.
33. Yang Q, Liu Y, Huang Y, Huang D, Li Y, Wu J, Duan M.Expression of COX-2, CD44v6 and CD147 and Relationship with Invasion and Lymph Node Metastasis in Hypopharyngeal Squamous Cell Carcinoma. PLoS One. 8(9),2013.
34. Yang X, Dai J, Li T, Zhang P, Ma Q, Li Y,Zhou J,Lei D. Expression of EMMPRIN in adenoid cystic carcinoma of salivary glands: correlation with tumor progression and patients' prognosis. Oral Oncol.46(10):755-60. 2010.
35. Zhang C,Man DP, Ma SM, Cao SW, Li DW. Expressions and significances of CD147, OPN and MMP-2 in oral squamous cell carcinoma.Sichuan Da XueXueBao Yi Xue Ban. 43(5):683-6, 2012.
36. Zhang Q,ZhouJ and KuXM, Chen XG *et al.* Expression of CD147 as a significantly unfavorable prognostic factor in hepatocellular carcinoma. Eur J Cancer Prev. 16(3): 196-202, 2007.
37. Zhao S, Ma W, Zhang M, *et al.*High expression of CD147 and MMP-9 is correlated with poor prognosis of triple-negative breast cancer (TNBC) patients.MedicalOncology. 30(1): 335, 2013.

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